Gene by Environment Effects in Arsenic Metabolism: Genetic Polymorphisms with Differential Effects in Children

The University of Arizona Superfund Basic Research Program

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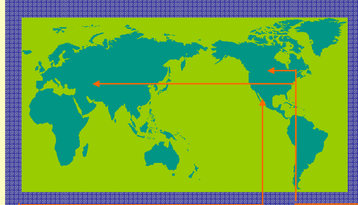
Abstract

For some time now, investigators have advanced the hypothesis that genetic determinants might underlie the individual variation in arsenic metabolism observed in numerous geographically diverse epidemiological studies. This could have important implications for disease susceptibility since arsenic metabolites have widely divergent toxic potencies, and signs of arsenicism have been associated with the distribution of urinary arsenic metabolites. The completion of the human genome sequence, together with new genetic experimental design approaches, have made comprehensively testing candidate genes for genetic association with arsenic metabolism a tractable problem.

Here we report the results of a genetic association study that evaluated three candidate genes for arsenic metabolism:

- glutathione-S-transferase omega-1 (GSTO)
- purine nucleoside phosphorylase (PNP)
- arsenic(3)-methyltransferase (AS3MT, formerly Cyt19)

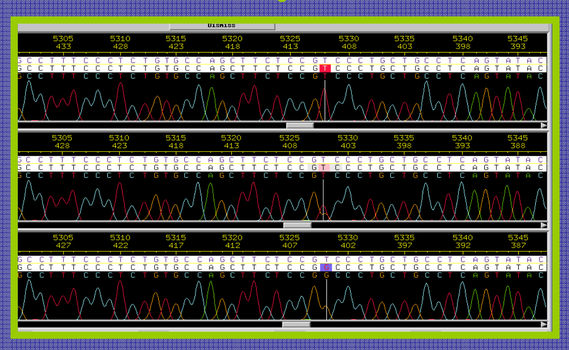
Methods



Polymorphism Catalogs: In order to determine the location of polymorphic sites within the genes, the genes were sequenced using DNA from 46 subjects, about half of European ancestry and half of indigenous American ancestry. DNA sequences from the 46 subjects were compared to identify positions within the genes at which some subjects had variant sequence.

Genetic Association Study: One hundred thirty five subjects from western Sonora, Mexico, exposed to drinking water arsenic (5–43 parts per billion) were evaluated for arsenic species in their first-morning void of urine. Two phenotypes were evaluated: the ratio of inorganic arsenic (III) to inorganic arsenic (V) (3/5 ratio) and the ratio of dimethyl arsenic (V) to monomethyl arsenic (V) (D/M ratio). Subjects' DNA was analyzed for polymorphisms in the three candidate genes.

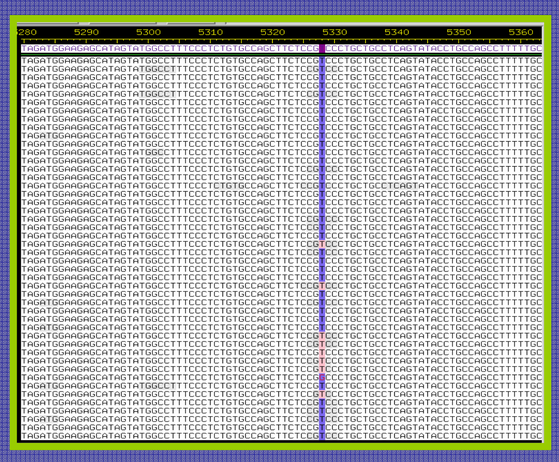
Generating Polymorphism Catalogs



At left, raw DNA sequence chromatograms from three human subjects for the gene PNP are shown. Because we carry two copies of PNP, the traces are a simultaneous readout of both copies, one inherited from each parent. In the highlighted polymorphic position, the person in the top trace has only the red "T" peak, and carries two copies of T. The person in the bottom trace has only the gold "G" peak, carrying two copies of G. The person in the middle trace has both peaks, and carries one copy of "T" and one of "G" at the polymorphic position.

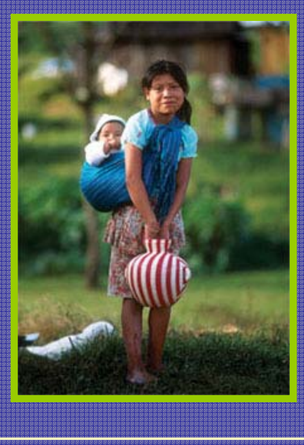
At right the same polymorphic position as displayed above in the trace plots is shown as lines of text, one line per human subject. As you can see, the majority of positions in the gene are identical among subjects. Within the polymorphic position, individuals with variant DNA sequences are tagged by pink boxes. This particular polymorphism also demonstrates ancestry specificity.

No European ancestry subjects are variant at this site, only the indigenous American ancestry subjects carry the variant DNA sequence, and in that group the variant is quite commonly found!



Results

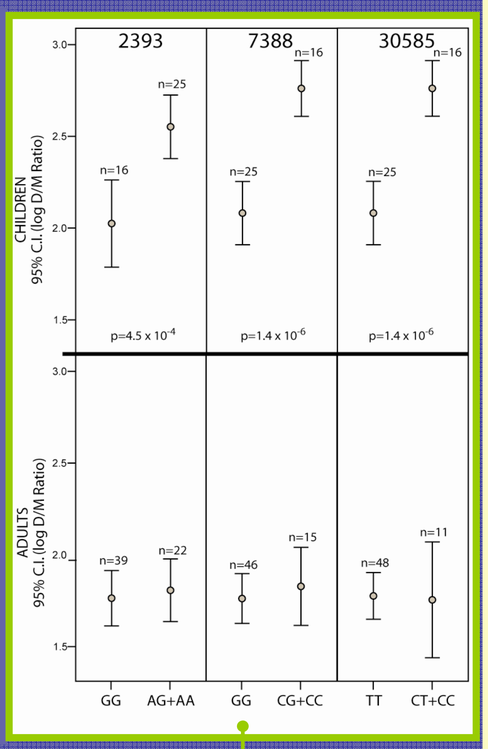
A total of 129 polymorphic sites were identified in the three genes. We employed a linkage disequilibrium-based algorithm to reduce the number of polymorphic sites to test for association with urinary arsenic metabolite levels to a total of 23 polymorphic sites. Two measures of urinary arsenic metabolites were used, DMA(V)/MMA(V) ratio and As(III)/As(V) ratio. Mean values for these were compared between subjects without any variant copies of the polymorphism and subjects with at least one variant copy of the polymorphism.



In the complete population of exposed Mexican subjects, AS3MT (Cyt19) variants are associated with urinary DMA/MMA ratio.

GENE	SITE	Phenotypes 3/5 Ratio	Phenotypes D/M Ratio
GST-O	-1859	N.S.	N.S.
GST-O	-1242	N.S.	N.S.
GST-O	5711	N.S.	N.S.
GST-O	8102	N.S.	N.S.
GST-O	8147	N.S.	N.S.
PNP	-1626	N.S.	N.S.
PNP	-1545	N.S.	N.S.
PNP	567	N.S.	N.S.
PNP	2934	N.S.	N.S.
PNP	3746	N.S.	N.S.
PNP	5837	N.S.	N.S.
PNP	6760	N.S.	N.S.
PNP	7821	N.S.	N.S.
CYT19	-400	N.S.	N.S.
CYT19	-262	N.S.	N.S.
CYT19	49	N.S.	N.S.
CYT19	2393	N.S.	0.024
CYT19	5207	N.S.	N.S.
CYT19	7388	N.S.	0.008
CYT19	7588	N.S.	N.S.
CYT19	8597	N.S.	N.S.
CYT19	20984	N.S.	N.S.
CYT19	30585	N.S.	0.003

t test (two-tailed), p values Bonferroni corrected for 46 tests



Surprisingly, the AS3MT genetic association with urinary DMA/MMA ratio is only observed in the children (7-11 yrs) and not in the adults (18-79 yrs.)

Conclusions

1. This study has replicated the findings of a previous study in Bangladesh demonstrating an age-specific elevation in urinary DMA(V)/MMA(V) ratios in arsenic-exposed children between about 5yrs and 12 yrs of age (Chowdhury et al. J. Environ. Sci. Health A38(1), 2003).

2. During this developmental window, it appears that the effects of genetic variation in the AS3MT gene are associated with particularly high DMA(V)/MMA(V) ratios, but this effect is lost once subjects age beyond this window.

Literature reports that suggest DMA(V)/MMA(V) ratio may be associated with skin lesions in arsenic-exposed people raise the possibility that these polymorphisms in AS3MT could be biomarkers of susceptibility, a hypothesis that awaits further testing. If validated, biomarkers such as the AS3MT variants can be used as modifying factors in formal risk assessments.

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